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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(21) International Application Number: PCT/US92/05592 (22) International Filing Date: 2 July 1992 (02.07.92) (30) Priority data: 725,983 5 July 1991 (05.07.91) US (60) Parent Application or Grant (63) Related by Continuation US 725,983 (CIP) Filed on 5 July 1991 (05.07.91) (71) Applicant (for all designated States except US): UNIVERSITY OF ROCHESTER [US/US]; 518 Hylan Building, Rochester, NY 14627-0140 (US).	(72) Inventors; and (75) Inventors/Applicants (for US only): VIOLANTE, Michael R. [US/US]; 15 Cedar Wood Circle, Pittsford, NY 14534 (US). PARKER, Kevin, J. [US/US]; 340 Howland Avenue, Rochester, NY 14260 (US). (74) Agents: MANSO, Peter, J. et al.; Ruden, Barnett, McClosky, Smith, Schuster & Russel, 200 East Broward Boulevard, P.O. Box 1900, Ft. Lauderdale, FL 33302 (US). (81) Designated States: AT, AU, BB, BG, BR, CA, CH, CS, DE, DK, ES, FI, GB, HU, JP, KP, KR, LK, LU, MG, MN, MW, NL, NO, PL, RO, RU, SD, SE, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LU, MC, NL, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, SN, TD, TG). Published <i>With international search report.</i>	

(54) Title: ULTRASMALL NON-AGGREGATED POROUS PARTICLES ENTRAPPING GAS-BUBBLES

(57) Abstract

Ultra-small, substantially non-aggregated, non-crystalline particles of predetermined uniform size which, when suspended in a liquid, contain entrapped gaseous bubbles are disclosed. These gaseous bubble particles are prepared by simultaneous co-precipitation of two compounds wherein one compound is substantially more soluble than the other in a given vehicle. When this vehicle is used for washing the co-precipitated particles, part of the soluble material is dissolved leaving a porous matrix. The porous particles then are dried and stored. The porous particles, which can be resuspended immediately prior to use, contain entrapped gas in the evacuated crevices or pores which is not displaced for a period of time because of surface tension of the suspending vehicle. The ultrasmall porous particles can be used as ultrasound contrast agents, such as ultrasound contrast agents in the blood vessels and soft tissue, including the liver, spleen, heart myocardium, kidney and brain of an animal, such as a human. The ultrasmall porous particles also can be used dry for applications where reduced weight is desirable.

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ULTRASMALL NON-AGGREGATED POROUS PARTICLES ENTRAPPING GAS-BUBBLESField of the Invention

The present invention relates to ultrasmall, substantially non-aggregated porous particles of predetermined uniform size which, when reconstituted contain entrapped gas bubbles. The ultrasmall porous particles are suitable for use as fillers and the like and as ultrasound contrast agents when suspended in a suitable ultrasound liquid. The present invention further relates to methods of making and using such ultrasmall, uniform porous particles.

Background of the Invention

Particles of compounds having low solubility in a dispersing medium are commonly used in a wide variety of applications, including pharmaceuticals, ceramics, paints, inks, dyes, lubricants, pesticides, insecticides, fungicides, fertilizers, chrom-

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atography columns, cosmetics, lotions, ointments, and detergents. Aqueous dispersions of particles are used in many cases to avoid hazards such as flammability and toxicity associated with organic solvents. Such dispersions typically have a broad range of particle size.

In many cases, product performance is improved by controlling the particle size distribution. In general, smaller particles of a compound provide a more uniform dispersion and will dissolve faster than larger particles of the same compounds. Control of particle size is, therefore, important in controlling the rate of solubilization.

Many drugs have been formulated as particles for sustained-release following oral, aerosol, subcutaneous, intramuscular, or other routes of administration. Particle size is one important factor affecting the release rate of these drugs. Those skilled in the art can discern other examples for using particle size to control product performance for the substances listed above.

Drugs that are insoluble in water can have significant benefits when formulated as a stable suspension of particles of less than three microns diameter. In this particulate form, the drug can be injected intravenously, circulate in blood, and be preferentially accumulated in, for example, the

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reticuloendothelial system, where it can facilitate normal reticuloendothelial functions such as detoxification. Alternatively, the drug can reside in the reticuloendothelial cells where it is stored until
5 solubilized or metabolized into an active form which circulates in blood to other tissues for efficacy. This "slow" release of active drug can provide more constant drug concentrations in plasma over a period of hours, days, weeks, or months, resulting in
10 improved therapeutic efficacy. Biodegradable particles which are radiopaque or labelled with a radioisotope are useful for diagnostic imaging of organs, such as liver and spleen, with high concentrations of fixed reticuloendothelial cells.

15 Solid biodegradable particles also can be useful for diagnostic ultrasound imaging of the liver and spleen with ultrasound. These particles can be effective if they have a density or compressibility significantly different from
20 surrounding tissue, thereby producing an impedance mismatch responsible for backscatter enhancement. Since tumors and other lesions generally do not contain these fixed reticuloendothelial cells, particles are not accumulated in these lesions so
25 only tissue parenchyma backscatter is enhanced creating a larger difference in echogenicity between parenchyma and lesion thereby facilitating lesion

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detection and diagnosis by ultrasound. See, for example, Parker, K.J. et al.: Ultrasound in Med. & Biol., 13(9): 555-566 (1987).

5

By far the simplest form of ultrasound contrast agents is free gas bubbles. Such bubbles may preexist in the liquid vehicle, or may be introduced via cavitation during the injection phase. Whatever the mechanism may be, it appears that many liquids, when rapidly injected into ducts or vessels, are capable of generating a quantity of air bubbles which may produce sufficient echoes to cause partial or complete intraluminal sonographic opacification.

10

The first report on the use of free gas bubbles appears to be that of Gramiak, R. and Shah, P.J.: Investigative Radiology, 3:356-366 (1968). They reported that they obtained anatomic validation of the aortic origin of cardiac echoes by means of direct physiological saline injection during continuous echocardiographic recording. The injection produced a cloud of echoes which was delineated by the parallel signal of the aortic root. See also, for example, U.S. Patent No. 4,276,885. Kremkau, F.W. et al.: Am. J. Roentgend., 3:159 (1968) also reported that they obtained intracardiac echoes from saline injection and from injection of autologous blood. They demonstrated that air bubbles may be

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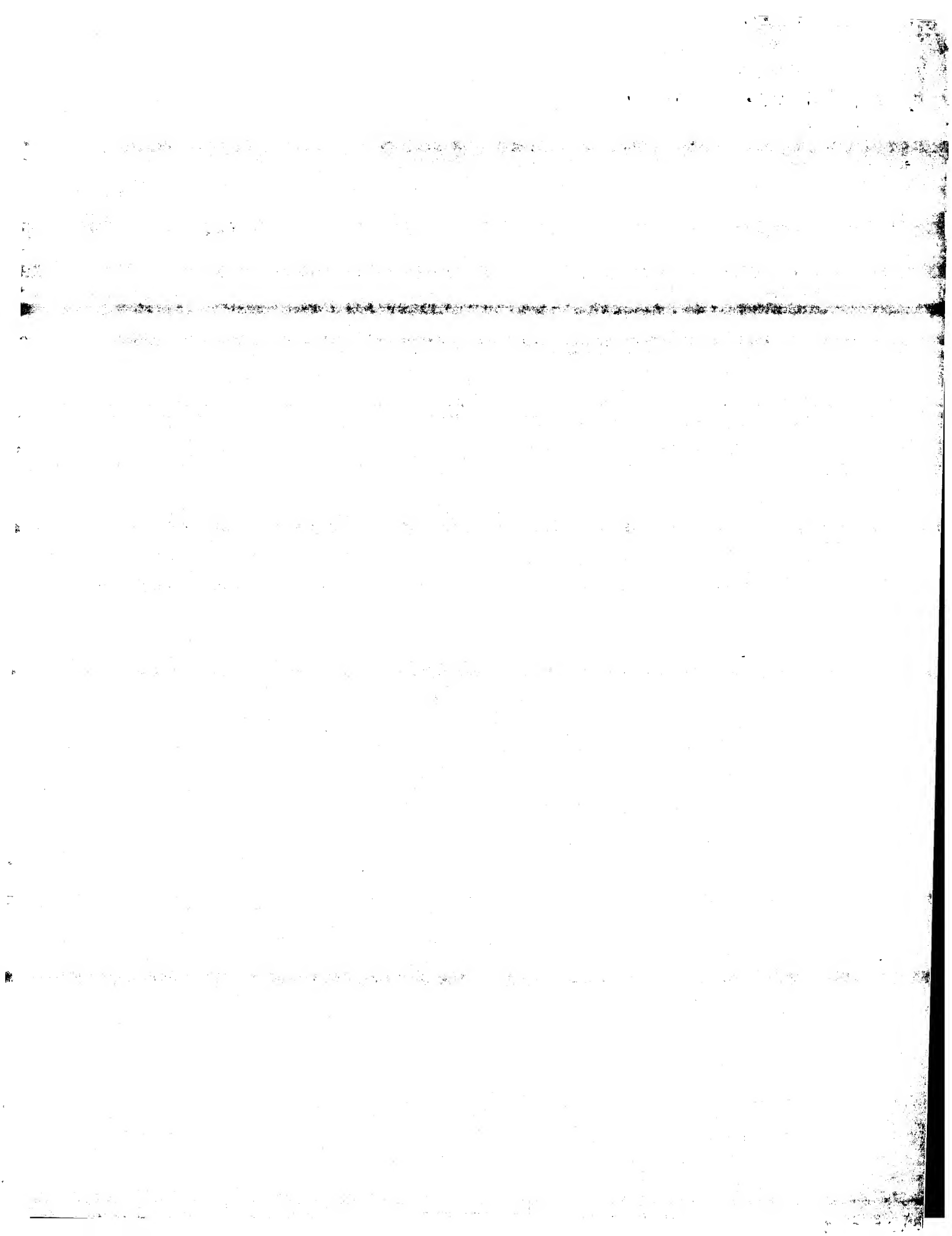
generated during the injection process itself. Zis-
kin, M.C. et al.: Investigative Radiology, 7:500-
505 (1972) reported using a variety of liquids, such
as renografin, carbonated water, and ether (which
5 boils at body temperature) to demonstrate the pre-
sence of echoes in all cases, detected by enhanced
Doppler signals from arteries. In recent years,
numerous investigators such as Chiang, C.W. et al.:
Chest, 89(5):723-726 (1986), Rizayev, M.N. and
10 Azatyan, T.S.: Heart J., 10(6):1308-1310 (1985),
Feinstein, S.B. et al.: J. Am. Coll. Cardiol.,
3(1):14-20 (1984), Konodo, S. et al.: J. Am. Coll.
Cardiol., 4:149-156 (1984), Ten-Cate, F.J. et al.:
J. Am. Coll. Cardiol., 3(1):21-27 (1984), Maurer, B.
15 et al.: Circulation, 69(2):418-429 (1984), Arm-
strong, W.F. et al.: J. Am. Coll. Cardiol.,
2(1):63-69 (1983), Tei, C. et al.: J. Am. Coll.
Cardiol., 3(1):39-46 (1984), Armstrong, W.F. et
al.: Circulation, 66(1):166-173 (1982), Meltzer,
20 R.S. et al.: Br. Heart J., 44(4):390-394 (1980a),
Meltzer, R.S. et al.: J. Clin. Ultras., 9(3):127-
131 (1981), Wise, N.K. et al.: Circulation,
63(5):1100-1103 (1983), Meltzer, R.S. et al.:
Ultrasound Med. Biol., 6(3):263-269 (1980b), have
25 reported the use of indocyanine green for opaci-
fication of the common bile duct in cholangio-
graphy. Presumably, microscopic air bubbles

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contained in the liquid or generated during the injection phase is responsible for the observed effects. Meyer-Schwickerath, M. and Fritzsche, T.: Ultraschall Med., 7:34-36 (1986) have reported urologic applications of a new commercial agent which incorporates solid particles as microbubble carriers.

While free gas bubbles are extremely efficient scatterers of sound energy, their utility is limited by the fact that they are effectively removed by the lungs or by pressure changes in the heart. Thus, it is impractical to use microbubbles to elicit contrast in the soft tissue via venous injection.

In an effort to overcome some of the limitations of free gas bubbles, encapsulated gas bubbles were manufactured and injected directly into the carotid artery in tumor bearing rabbits. See Carroll, B.A. et al.: Investigative Radiology, 15(3):260-266 (1980). These consisted of nitrogen gas trapped in 80 micron gelatin capsules. Carroll et al. report ultrasonic enhancement of tumor rims in rabbits with VX2 carcinoma. The large size of these particles did not allow their administration in the peripheral circulation. Unfortunately, the manufacturer of small (2-3 microns), gas filled capsules which could clear the lungs is difficult due to the extreme



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thinness of the capsule wall through which gas diffuses.

As another alternative, U.S. Patents, No. 4,442,843, No. 4,657,756 and No. 4,681,119, disclose the use of aggregates as carriers of gas to produce microbubbles in blood to alter the transmission characteristics thereof to electromagnetic and sonic waves transmitted therethrough. Unfortunately, because the aggregates are of such a large size, i.e., on the order of between about 20-250 microns, it is difficult if not impossible for such aggregates to travel past the lungs and heart, thereby limiting their usefulness as ultrasound contrast agents. In addition, because the solid materials from which the aggregates are formed will generally solubilize in body fluids over a short period of time, their ability to enhance ultrasound images in the lungs and heart is short-lived. Moreover, the individual particles from which the aggregates are formed have little bubble generating capacity in their unaggregated form.

Consequently, there is a demand in the ultrasound industry for a contrast agent which offers enhanced gas bubble echogenicity with good long-term stability for arterial and organ ultrasound image enhancement following intravenous injection.

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Summary of the Invention

In brief, the present invention alleviates and overcomes certain of the above-mentioned problems and shortcomings of the present state of the art through the discovery of novel ultrasmall, substantially non-aggregated, non-crystalline porous particles of substantially uniform size which, when reconstituted, contain entrapped gas bubbles, and methods of making and using same.

The novel ultrasmall, non-aggregated porous particles of the instant invention are uniquely suited for use as ultrasound image enhancers and for ultrasound measurements, such as Doppler techniques, when reconstituted with suitable physiologically acceptable liquids. Quite amazingly, the novel ultrasmall non-aggregated porous particles provide unique gas bubble echogenicity with good long-term stability for arterial and organ ultrasound image enhancement following intravenous injection. Even more amazingly, the ultrasmall porous particles of the instant invention are able to accomplish this without having to form aggregates in order to develop microbubbles to enhance ultrasound imaging.

It has also been discovered, and quite surprisingly, that the novel ultrasmall, non-aggregated porous particles of the instant invention produce enhanced and long-term ultrasound back

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scatter as a result of gas bubbles, such as air or helium bubbles, being trapped within their solid matrices or pores when they are suspended in a liquid. It is believed that the solid matrices or pores provide the stability for the unique sustained echogenicity which heretofore has not been achieved by the ultrasound contrast agents presently available. And, because the novel ultrasmall porous particles of the instant invention have virtually no tendency to aggregate, they are uniquely suited for use as contrast agents to enhance ultrasound images in the blood vessels and soft tissue or organs throughout an animal. In other words, because the novel ultrasmall porous particles have the capacity to circulate throughout the body of an animal, their suitability as ultrasound contrast agents greatly extends beyond the lungs and heart into, for example, the liver, spleen, heart myocardium, kidney, brain and the like.

In accordance with the present invention, the ultrasmall, porous particles of the instant invention are believed to be stable at ambient temperatures, have little to no tendency to aggregate, and are non-toxic and physiologically acceptable when introduced into the bloodstream of living beings, such as humans. The size of the ultrasmall, porous particles of the present invention typically

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range up to about 10 microns, and preferably from about 0.01 microns to about 5.0 microns, and more preferably from about 0.1 microns to about 2.0 microns.

5 Preferred ultrasmall, substantially non-aggregated, non-crystalline porous particles of the instant invention are iodipamide ethyl ester particles having a substantially uniform mean diameter on the order of, for example, about 0.5 up to about 2
10 microns and the ability to entrap gas bubbles within their pores after resuspension in a liquid vehicle.

In accordance with a further feature of the instant invention, the ultrasmall porous particles of substantially uniform size are made by, first,
15 preparing a solution of two separate solid compounds in a suitable solvent for the two compounds, second, infusing a precipitating liquid into the solution at a temperature between about -50°C and about 100°C and at an infusion rate of from about 0.01 ml/min.
20 to about 3000 ml/min. per unit volume of 50 ml, the two solid compounds having essentially little solubility in the precipitating liquid and the solvent being miscible in the precipitating liquid, so as to produce a suspension of precipitated solid compounds
25 in the form of substantially non-aggregated particles with a substantially uniform mean particle diameter selected from the range of up to about 10

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microns, such that the particle size is directly related to the solution temperature and inversely related to infusion rate, third, separating the co-precipitated particles from the solvent, and, fourth, washing the co-precipitated particles with a washing liquid which serves to selectively solubilize and remove the second compound as well as any remaining residual solvent thereby producing particles consisting of a porous matrix consisting of only, or mostly, the first compound. For example, when producing ultrasmall porous particles, such as ultrasmall porous iodipamide ethyl ester (IDE) particles, in accordance with the instant invention, the IDE porous particles formed after the washing step are believed to be mostly IDE, but the matrix of the IDE particles may include, for instance, some iodipamic acid (IDA). The ultrasmall "porous" particle suspension is then exposed to gas, for example, at increased pressure, or dried to remove as much moisture as possible and to permit the porous particles to entrap gas within their porous matrices upon suspending the particles in liquid vehicles. The ultrasmall porous particles of the instant invention are much less dense than pure solid particles formed alone following the same procedure.

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It should be appreciated by those versed in this art that when the dried ultrasmall, porous particles are reconstituted or suspended in a suitable liquid, they are completely redispersed, but
5 now a small amount of air or other gas such as helium has been entrapped in the particle crevices or pores where the second compound initially was present. The entrapped air, crevices or pores is believed to remain for several hours, even after
10 resuspension in a liquid vehicle. Moreover, it is believed that because the crevices or pores are so small, the surface tension of the suspending liquid does not permit rapid filling. Consequently, such reconstituted suspensions are uniquely suited for
15 use as ultrasound contrast materials because the echogenic gas bubbles trapped within the solid porous particles are stabilized, even against pressure changes in the heart which typically destroys other competitive ultrasound contrasting agents
20 currently available.

In practicing the methods to produce the ultrasmall porous particles of the instant invention, a preferred weight ratio of the more soluble to the less soluble compound in the washing solution
25 is from about 2:1 up to about 10:1. In addition, the amount of the more soluble compound dissolved and removed during the washing of the particles can

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be from about 10% to about 100% of the amount of the more soluble compound present in the particles after precipitation, but before washing.

5 In one preferred embodiment, the less soluble compound in the washing solution is iodipamide ethyl ester (IDE) and the more soluble compound in the washing solution is iodipamic acid (IDA). The precipitating liquid is water at about pH 5 and the washing liquid is aqueous 0.1% PVP at
10 about pH 11. When washing the co-precipitated particles in accordance with the methods of the instant invention, washing can be continued until most or all IDA has been removed but most or all of the IDE remains which, for example, is preferably at
15 a pH of about 3.3 to about 3.4 when producing ultrasmall porous IDE particles. When washing is stopped at a pH of about 3.3 to 3.4, it is believed that the yields and echogenicity of the ultrasmall porous IDE particles are enhanced.

20 In accordance with a further feature of the present invention, the ultrasmall, porous particles may be coated with various substances, such as human serum albumin or selected antibodies, to alter the surface properties of the particles to improve, for
25 example, their biocompatibility or their ability to target a desired site.

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Accordingly, it can now be appreciated by those versed in this art that the present invention provides a solution to the ultrasound art that has long sought to overcome the shortcomings associated with the ultrasound contrast agents and methods available heretofore.

The above features and advantages of the present invention will be better understood with reference to the FIGS., Detailed Description and Examples set out hereinbelow. It will also be understood that the ultrasmall, porous particles and methods of this invention are exemplary only and are not to be regarded as limitations of this invention.

Brief Description of the FIGS.

Examples of the present invention will now be more fully described, with reference to the accompanying FIGS., wherein:

FIG. 1 depicts raw backscatter values (RMS of in vitro bubble/IDE particle suspensions at 5.2 mg/ml plotted versus the weight ratio of iodipamic acid (IDA) to iodipamide ethyl ester (IDE) in a formulation mixture before dissolving away the IDA.

These data demonstrate increased echogenicity (porosity) with higher ratios of IDA/IDE;

FIG. 2 depicts raw backscatter values (RMS) of in vitro bubble/IDE particle suspensions at 5.2,

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9.1 and 13.1 mg/ml plotted versus time after mixing with bovine plasma. These data demonstrate increased backscatter with increased particle concentration. These data also demonstrate the extremely long persistence of this backscatter after mixing with bovine plasma compared with other bubble agents which survive only seconds to minutes;

FIG. 3 depicts raw backscatter values (RMS) of in vitro bubble/IDE particles at 5.2 mg/ml and standard solid IDE particles at 12.2 mg/ml plotted versus time. These data demonstrate the increased echogenicity obtained with bubble/IDE particles compared with that for solid IDE particles. This enhanced echogenicity is observed even though the bubble/IDE particles are at less than half the concentration of the solid IDE particles;

FIG. 4 depicts B-scan image at 5 MHz of normal rabbit liver with gall bladder before infusion of the bubble/IDE contrast agent;

FIG. 5 depicts B-scan image at 5 MHz of normal rabbit liver with gall bladder, but 60 minutes following intravenous infusion of the bubble/IDE contrast agent. The enhancement of the parenchymal echogenicity was observed for at least 120 minutes after contrast administration;

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FIG. 6 is a graph of infusion rate (ml/min.) (of aqueous precipitating liquid) as a function of the product of stir rate (rpm) and total volume (liters) of the organic solution at a constant temperature; the relationship: aqueous infusion rate (ml/min.) = $23 + 0.14 [\text{stir rate (rpm)} \times \text{volume organic solution(l)}]$ defines the parameters for production of iodipamide ethyl ester particles of one micron diameter at a constant temperature (4°C) and in dimethylsulfoxide/ethanol;

FIG. 7 is a graph showing iodipamide ethyl ester particle size as a function of temperature at a constant ratio of infusion rate of aqueous precipitating liquid to $[\text{stir rate (rpm)} \times \text{volume of organic solution}]$;

FIG. 8 is a graph demonstrating the effect on particle size of varying the infusion rate of aqueous precipitating liquid at constant temperature and stirring rate of an iodipamide ethyl ester solution; and

FIG. 9 depicts raw backscatter values (RMS) of in vitro bubble-IDE particle suspensions at 5.2 mg/ml. These data demonstrate that backscatter increases linearly with concentration.

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Detailed Description of the Invention

By way of illustrating and providing a more complete appreciation of the present invention and many of the attendant advantages thereof, the following detailed description is provided concerning the novel ultrasmall, substantially non-aggregated, non-crystalline porous particles, and methods of making and using such particles.

This invention concerns the preparation of non-aggregated porous particles of a predetermined uniform size. One aspect of the invention concerns the preparation of uniform particles of a predetermined size in a vehicle in which the concentration of the compound in the vehicle is greater than the solubility of the compound in that vehicle. The particles are formed by a carefully controlled precipitation of the compound into a suitable precipitating liquid from a solvent in which the compound is soluble.

A second aspect of this invention is the simultaneous co-precipitation of two compounds having significantly different solubilities in designated washing solutions such that the more soluble compound can be dissolved and removed from the less soluble compound during the washing of the co-precipitated particles. The porous particles then can be coated to change the surface properties

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if so desired. The porous particles can then be dried to remove almost all moisture from the particles leaving porous particles with lower density. Such particles in dry form can be useful for high strength low weight materials.

Particles which are about one micron diameter or smaller have pores so small that they do not fill rapidly with liquid when the particles are rewetted. In this way it is possible to entrap air (or other gas) in the particles. Such particles can be useful as echogenic ultrasound contrast materials.

An important principle underlying this invention is the differential solubilities of the two compounds chosen for co-precipitation. For example, an ester and the acid from which it is synthesized may both be insoluble in aqueous solutions at, for example, pH 5-7. However, the acid may have significantly higher solubility at pH 10-11 such that washing at basic pH dissolves the acid leaving porous particles consisting solely, or mostly, of the ester compound. Other acid derivatives, such as amides can be substituted for the ester compound recognizing that the wash solution may have to be at a lower pH to be effective.

Similarly, two compounds could be chosen for washing with an organic solvent as long as the

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two compounds have significantly different solubilities in that solvent.

This invention can be practiced utilizing compounds which differ in solubility in a given wash solution by orders of magnitude. The difference in solubility, however, can be small - a factor of two or less - but washing conditions must be adjusted to create the porous particles.

Varying the ratio of more-soluble to less soluble components can alter the properties of the resultant particles. For example, varying the ratio of iodipamic acid (IDA) to iodipamide ethyl ester (IDE) from 2:1 to 8:1 significantly increases the echogenicity of the resultant particles, as shown in FIG. 1. These data illustrated in FIG. 1 were acquired at 4.7 mg/ml.

Varying the concentration of particles also affects the echogenic properties of a suspension. As shown in FIG. 2, the RMS backscatter of particles in plasma increased from 0.4 to 1.2 when the concentration of particles increased from 5.2 to 13.1 mg/ml.

The ultrasmall porous particles of the instant invention can be coated with substances to alter their surface properties. For example, coating the particles with serum albumin can improve the biocompatibility of such particles. Coating with

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antibodies may improve the targeting of particles to a desired site. Coatings altering the wettability, charge, or permeability of the particle surface of such particles may be applied prior to drying to achieve the desired surface characteristics.

Drying can be accomplished by any of a number of techniques known to one skilled in the art. Vacuum, spray, or lyophilization may be utilized depending on the compound(s) and application for these particles.

After drying, the particles can be reconstituted with any liquid in which the particles are not soluble. For ultrasonic contrast agents, the liquid generally will be water or some other suitable aqueous solution. Other liquids may be appropriate for other applications.

Methods other than drying can be utilized to introduce gas into the porous particles. For example, supersaturation and rectified diffusion under acoustic irradiation can utilize the porous particles as nuclei for gas cavity formation in situ.

The preferred IDE/IDA particles are believed to be useful for diagnostic imaging with both computer tomography (CT) and ultrasound. The iodine in the IDE solid matrix is the effective attenuator for CT while the entrapped air is the effective scatterer for ultrasound. Similar particles could

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be useful for magnetic resonance imaging as well. This would be possible by reconstituting the dried particles with a liquid containing a paramagnetic material, such as gadolinium chelates. After allow-

5 ing sufficient time for equilibration of the paramagnetic material into the pores of the particles, the particles could be useful for enhanced magnetic resonance imaging of tissues, e.g. liver, in which the biodistribution of the entrapped paramagnetic

10 material is controlled by the particles rather than the bulk liquid biodistribution. It is ~~is~~ believed that this can be accomplished by suspending the dried porous particles in a paramagnetic material, such as gadolinium EDTA (Gd EDTA). For example,

15 once the porous particles have been suspended in gadolinium EDTA, they can be introduced into an animal in an effective amount such that the porous particles and Gd EDTA will accumulate in the phagocytic cells in the liver, while the remaining

20 Gd EDTA will distribute normally and clear quickly from the animal. The Gd EDTA accumulated in the liver will remain in the phagocytic cells for a period of time to enhance the liver parenchyma during imaging. Tumor and metastatic cells will,

25 however, not be enhanced upon imaging thereby improving their detection. Other combinations,

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e.g., diagnostic and therapeutic agents, could be utilized in the practice of this invention.

5 The physical chemical principles thought to be involved in this invention suggest that the free energy of the system is higher when the compound is dissolved in the organic solvent than when the compound exists in the particulate or crystalline state. During precipitation the compound will naturally convert to the crystalline form - the
10 lowest free energy state - unless it is trapped in the metastable particulate form, a condition where its free energy is intermediate between the solution and the crystalline phases. When properly practiced, this invention enables the trapping of the
15 compound in the metastable particle state, precluding transformation to the crystalline state.

The size distribution of particles formed during co-precipitation can be correlated with the time interval between onset and completion of co-precipitation. It is believed that a very short
20 time interval results in the production of uniformly sized particles, while a very long time interval results in a broad particle size distribution. Intermediate conditions produce intermediate particle size distributions.
25

An important parameter for utilization of this invention is the solubility of the compounds in

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the precipitating liquid. Thus, compounds having essentially little aqueous solubility, i.e., compounds which have an aqueous solubility of less than one part in ten thousand, may be precipitated in an aqueous solution in order to obtain an excellent yield. Compounds which are more water-soluble can also use an aqueous precipitating liquid. However, the higher the solubility of the compounds, the greater the probability that some of the compounds will dissolve in the aqueous phase and transform to the more stable crystalline state. Also, redissolution in the aqueous phase can lead to a broadening of the particle size distribution. For these reasons, it is preferred that an aqueous precipitating liquid be used for compounds having a water-solubility of less than one part in ten thousand.

It has been found that it is possible to prepare suspensions of compounds which are poorly soluble in aqueous solutions, i.e., have a solubility from about one part per ten thousand to about one part per one hundred which provide excellent yields by using an acceptable precipitating liquid in which the compounds have even less solubility than water. The difference in the solubility of the compounds in water as compared to the precipitating liquid need not be large in order to be significant in terms of

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yield. In order to make particles of a uniform and predetermined size, a solution of the solid compounds in a suitable solvent is prepared. The solution may be diluted with a non-solvent that does not cause the compounds to precipitate. A precipitating liquid is also prepared, preferably with a surfactant, in sufficient quantity to both coprecipitate the compounds and stabilize the resulting suspension of particles of the compounds against aggregation. The precipitating liquid may be used alone when compounds which do not aggregate are used. The precipitating liquid is infused into the solution in which the compounds are dissolved under carefully controlled conditions, including: the rate of stirring of the organic solution, the rate of infusion of the aqueous solution, the volume of the organic solution and the temperature of the solutions and the suspension. The precipitating liquid may be infused, for example, through a needle of standard gauge.

In investigations of varying parameters to adjust for particle size, three usable relationships were discovered: (1) diluting the solution with more of the non-solvent produces larger particles, and diluting with less of the non-solvent produces smaller particles; (2) higher temperatures of the

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solution during precipitation produce larger particles, and lower temperatures of the solution during precipitation produce smaller particles; and (3) at a given stirring rate of the organic solution, faster infusion rates of precipitating liquid produce smaller particles while slower infusion rates produce larger particles.

When the co-precipitation is complete, the uniformly sized particles are washed to remove the solvent, i.e. by centrifugation, filtration, etc, and the soluble compound to produce the porous particles of the instant invention. In most cases, the particles should be separated from the solvent quickly to prevent transformation to a crystalline form.

Aqueous precipitating liquids are useful for many compounds, including but not limited to organic compounds such as iodipamide ethyl ester, iothalamate ethyl ester, ioesefamate ethyl ester, 2,2', 4 4'-tetra-hydroxybenzophenone, RS nitro-cellulose, progesterone, beta-2,4,6-triiodo-3-dimethyl formamidinophenyl propionic acid ethyl ester, N-(trifluoroacetyl) Adrimycin 14 valerate, 1,2 diaminocyclohexane malinate platinum (II), norethisterone, acetyl salicylic acid, wafarin, heparin-tridodecyl methyl ammonium chloride complex, sulfamethoxazole, cephalixin, prednisolone acetate,

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diazepam, clonazepam, methidone, naloxone, disulfiram, mercaptopurine, digitoxin, primaguine, mefloquine, atropine, scopolamine, thiazide, furosemide, propranolol, methyl methacrylate, poly methyl methacrylate, 5-fluorodeoxyuridine, cytosine arabinoside, acyclovir, and levonorgestrel; and inorganic compounds such as aluminum chloride hexahydrate, the oxides of iron, copper, manganese and tin.

Compounds which are better suited for precipitation using a non-aqueous precipitating liquid include organic compounds such as mitindomide, hydrolytically unstable compounds such as isopropylpyrrolizine (IPP, or carbamic acid, (1-methylethyl)-, (5-(3, 4-dichlorophenyl)-2, 3-dihydro-1, H-pyrrolizine-6-, 7-diyl) bis(-methylene ester); and inorganic compounds such as iron citrate, iron iodate, calcium pyrophosphate, calcium salicylate, platinum dichloride and sodium pyrophosphate.

The first step is to prepare a solution of two compounds, one compound being of interest, in a suitable solvent for the compounds. This can occur by simply dissolving the compounds in the solvent of choice.

The solvent is chosen to suit the compounds. For example, dimethylformamide (DMF) is a

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solvent for iothalamate ethyl ester (IEE) and
iosefamate ethyl ester (IFE), and dimethylsulfoxide
(DMSO) is a solvent for iodipamide ethyl ester (IDE)
and IEE. DMSO is also a suitable solvent for com-
pounds such as mitindomide. Another suitable
solvent for many compounds, and especially IPP, is
tetrahydrofuran (THF).

The solution is then optionally diluted
with a non-solvent that does not cause the compounds
to precipitate. The non-solvent causes greater
dispersion of the dissolved molecules of the com-
pounds in the liquid phase. Greater dilution of the
solution with non-solvent produces larger particles,
and less dilution of the solution with non-solvent
produces smaller particles.

The non-solvent should not precipitate the
compounds when it is added to the solution. Lower
aliphatic alcohols, such as ethanol, are effective
non-solvents for solutions of IDE and IEE in DMSO.
For the ethyl esters of triiodobenzoic acid, propor-
tions of non-solvent to solvent at a ratio of 2 or
more can produce 1 to 3 micron sized particles
(depending on other parameters); and ratios of less
than 2 can produce submicron particles, at least as
applied to DMSO solutions diluted with ethanol.

To co-precipitate the compounds from the
solution in a desired particle size, preferably a

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solution of a surfactant is prepared in sufficient quantity to effect complete precipitation of the compounds and to stabilize the resulting suspension of particles of the compound against aggregation.

5 The surfactant provides the stabilization against aggregation, while a suitable precipitating agent causes the co-precipitation of the compounds. Presence of extra surfactant solution is advisable to ensure stabilization so that the co-precipitated

10 particles suspended in liquid do not aggregate, forming agglomerates of an improperly large size. While surfactants are used in most cases, some compounds appear to form stable, substantially non-aggregated particles without the use of surfactants.

15 Examples of such non-aggregating compounds are certain heparin complexes.

It is thought that particles with relatively high surface charge are less likely to require surfactant in the precipitating solution.

20 The surface charge of a particle is sometimes referred to as its zeta potential, a measurement of charge which falls off with distance. There may be a threshold zeta potential above which no surfactant is needed, but below which, surfactant is needed to

25 keep the precipitating particles from aggregating. The zeta potential is directly correlated with the polarity or net charge of a compound. Thus, the

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need for surfactant in the precipitating solution may be predicted from the extent of the charge or polarity of the compound employed in the method of the invention. For example, heparin complexes are highly charged, and form stable non-aggregated particles when precipitated with water.

Generally, such a theory notwithstanding, empirical methods will suffice; that is, a co-precipitation may first be performed with water, and if aggregation occurs, then a co-precipitation in the presence of surfactant is indicated. Surfactants are chosen for their compatibility with the compounds and their ability to stabilize a suspension of compound particles. For work with IEE and IDE drugs, a solution of 5% polyvinylpyrrolidone (C-30), 0.1% poly-vinylpyrrolidone (C-15), or 0.1% human serum albumin is preferred. Also 0.1% Pluronic F-68, [Poloxamer 188, a poly(oxyethylene-co-oxypropylene) polymer], a 0.33% gelatin, 0.33% gelatin plus 0.6% Hetastarch, 0.33% gelatin plus 0.002% propylene glycol, and 0.3% gelatin plus 2% sucrose, or other surfactants known to one skilled in the art can be used.

To co-precipitate particles of the compounds in the desired sizes, the precipitating liquid and the solution are combined under controlled conditions of temperature, ratio of

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infusion rate to stirring rate, and the proportion of non-solvent to solvent in the dispersed solution.

Preferably, the solution being infused with precipitating liquid is agitated. This can be accomplished by stirring, shaking, by the infusion itself and by other techniques known to those skilled in the art. This effect can also be achieved by combining a stream of precipitating liquid with a stream of the solution.

The co-precipitation of the compounds occurs exothermically, heating the solution and the resulting suspension. The temperature of the solution and resulting suspension is controlled to achieve the particle size of precipitate that is desired. Higher solution temperatures during precipitation produce larger particles, and lower solution temperatures during precipitation produce smaller particles. Since many compounds are less soluble at lower temperatures, it is generally preferred to conduct the infusion of precipitating liquid at a low temperature in order to maximize yield. The lower limit of the temperature at which co-precipitation can be conducted is, of course dependent upon the freezing point of the solvent, precipitating liquid, as well as economic concerns.

Also, faster infusion rates at constant stirring rate of organic solution produce smaller

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particles, and slower infusion rates produce larger particles.

FIGS. 6-8 show the effects on particle size of varying parameters during precipitation of IDE from a DMSO solution diluted with 1 part solution to 2 parts ethanol using an aqueous solution of 5% polyvinylpyrrolidone at different infusion rates and temperatures.

FIG. 6 shows that as the volume and stirring rate of the organic compound iodipamide ethyl ester and dimethyl sulfoxide/ethanol solution are increased, the infusion rate of aqueous surfactant solution must be increased proportionally as defined by: infusion rate (ml/min.) = $23 + 0.14$ [volume (liters) X stir rate (r.p.m.)] to produce particles of 1 micron diameter at 4°C.

FIG. 7 shows that at a constant ratio of infusion rate to [stir rate X volume], increased precipitation temperature produces larger particles.

FIG. 8 plots 3 points for rate of infusion of the precipitating liquid into the organic solution to approximate the curve by which larger particles are formed from slower injection rates, showing that at a constant ratio of temperature to [stir rate X volume], particle size is inversely related to the rate of infusion of the precipitating liquid.

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When FIGS. 6-8 are considered together, they show clearly that higher temperatures and slower mixing rates produce larger particles, and lower temperatures and faster mixing rates produce smaller particles. Another parameter than can be varied to affect particle size is the amount of dilution of the solution before co-precipitation occurs.

When the co-precipitation is complete, extra surfactant solution can be added to further stabilize the suspended particles against agglomeration. The extra solution can be added at a rapid rate, since essentially all the compounds are now co-precipitated in uniformly sized particles. The precipitated particles are promptly separated from the solvent to prevent redissolving and reprecipitation of particles at undesirable sizes. Centrifuging is a preferred way to perform the separation. Other methods, including membrane filtration, reverse osmosis, and others known to persons skilled in the art may also be used to remove undesired substances.

Promptly after separating the particles, the particles are washed or rinsed or titrated with a solution in which one of the two compounds initially dissolved is soluble to remove solvent and excess surfactant and to remove or extract such

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solubilized compound from the precipitated particles and dried as discussed hereinabove.

5 The dried porous particles prepared according to the method outlined above may be resuspended in an appropriate suspension vehicle which may be aqueous or non-aqueous solution, as the situation requires. For example, where the porous particles formed comprise a pharmaceutical compound for parenteral administration, the porous particles are
10 ultimately resuspended in an aqueous solution, such as a sterile saline solution. In so doing,—however, gas bubbles, such as air bubbles, will be entrapped in the crevices or pores of the particles. In other instances, the particles may be suspended in a
15 carrying agent such as an ointment, gel, or the like. Preferably, the compound has the same range of solubility in the suspension vehicle as in the precipitating liquid.

20 It should be understood to those versed in this art that the methods and ultrasmall, non-aggregated solid particles disclosed in U.S. Patents, No. 4,826,689 and No. 4,997,454, provide teachings upon which the instant invention has improved, and therefore are incorporated herein by
25 reference in their entireties.

 Examples of ultrasmall, substantially non-aggregated porous particles of the present

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invention will now be further illustrated with reference to the following examples.

Example I

5 Ultrasmall, non-aggregated porous
iodipamide ethyl ester (IDE) particles having a
substantially uniform mean diameter of about 0.5
microns were prepared as recited hereunder and
suspended in bovine plasma-distilled water (1:1)
solution and placed in a small plastic pipette.

10 A pulse echo technique was used to
determine relative backscatter. A wide band 10 MHz
center frequency, Panametrics transducer (1.3 cm
diameter 5 focus), driven by a JSR Pulser, was used
to obtain rf scan lines. For in vitro measurements,
15 the mean backscatter (root mean square, RMS) was
computed for eight uncorrelated scan lines, each
corresponding to about 4 mm in length.

In vitro analysis of the bubble/IDE par-
ticle suspension reveals that backscatter increases
20 monotonically with concentration, as shown in FIG. 9.

In vitro analysis of the bubble/IDE par-
ticle suspension and a standard solid IDE particle
suspension after mixing with bovine plasma, reveals
a much higher backscatter from the bubble/IDE
25 particles than from the solid IDE particles, as
shown in FIG. 3. Moreover, these data demonstrate

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that the high echogenicity of the bubble/IDE particles is sustained for hours after mixing with bovine-plasma.

To prepare the bubble/IDE suspension, about
5 250 mg of solid iodipamide ethyl ester (IDE) and 500
mg of iodipamic acid (IDA) were added to a 50 ml
beaker with 1" x 5/16" teflon coated stir bar.
Approximately 5 mls of dimethylsulfoxide (DMSO) were
added and stirred for about 10-15 min. until dis-
10 solved. To the solution, approximately 6.25 mls of
absolute ethanol were added. The beaker was then
cooled in an acetone/dry ice bath. While cooling,
the mixture was stirred quickly, but without
splashing. Approximately 4.5 mls of water at about
15 pH 5 in about 0.5 ml increments were slowly injected
into the mixture while maintaining the temperature
thereof at about 0°C. Approximately 8 mls of water
at about pH 5 was then infused into the mixture at a
rate of about 5 mls/min. starting at about 0°C.
20 Thus, a total of 12.5 mls of water was infused into
the mixture. A Harvard Infusion pump and a 60 cc
plastic syringe and 19 gauge infusion set (butter-
fly) were used to infuse the water. Co-precipita-
tion occurred after about 6.5 mls of water were
25 infused into the mixture and at a temperature of
about 1°C. Following co-precipitation, the
suspension was stabilized by adding about 150 mls of

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1% polyvinylpyrrolidone (PVP) at about pH 7.4 and kept at a temperature of about 10°C. The suspension was then poured into a 250 ml centrifuge bottle (approximately 31.25 mls) and centrifuged at approximately 2500 rpm for about 30 min. The supernate was discarded. The precipitate in the centrifuge bottle was then repeatedly washed with approximately 31.25 mls of about 0.1% PVP at about pH 11 until the wash reached a pH of about 10 to extract the IDA from the IDE particles to generate the ultrasmall, non-aggregated, porous, non-crystalline IDE particles. The particles were then treated with human serum albumin according to Example III and then lyophilized according to the procedure set forth in Example IV.

Example II

New Zealand white rabbits (Hazelton Laboratories) weighing 2-4 Kg were anesthetized, and injected intravenously at a rate of about 1 ml/min. with a 8-10 ml (depending on rabbit weight) suspension of iodipamide ethyl ester (IDE) porous particles having a substantially uniform mean diameter of about 0.5 microns. The final concentration of the bubble/IDE particle suspension was approximately 100 mg/ml. The injected dose of the bubble/IDE particle suspension was approximately 250 mg IDE/Kg

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rabbit body weight. The IDE particles contained air bubbles trapped within their crevices or pores.

5 The rabbits were scanned periodically. B-scan images of rabbit liver with and without the bubble/IDE particle suspension are shown in FIGS. 4 and 5. These images were obtained from a 5.0 MHz Acuson Scanner with all settings held constant over a 120 min. examination.

10 Liver echogenicity following intravenous administration of the bubble/IDE particle suspension is markedly enhanced as compared with no contrast agent, as evidenced by FIGS. 4 and 5. Results in these rabbits demonstrate that the stabilized/

15 echogenic gas can be delivered to the liver, raising the backscatter well above the levels obtained with no contrast agent or with standard solid IDE particles. Improving liver lesion detection by enhanced ultrasound may now be possible with the bubble/IDE particles.

20 To prepare the bubble/IDE particle suspension, about 5000 mg of solid iodipamide ethyl ester (IDE) and about 10,000 mg of iodipamic acid (IDA) were added to a 800 ml beaker with 2" X 5/16" teflon coated stir bar. Approximately 100 mls of dimethyl-

25 sulfoxide (DMSO) were added and stirred for about 10-15 min. until dissolved. To the solution, approximately 125 mls of absolute ethanol were

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added. The beaker was then cooled in an acetone/dry ice bath. While cooling, the mixture was stirred quickly, but without splashing. Approximately 90 mls of water at about pH 5 at about 10 ml increments were slowly injected into the mixture while maintaining the temperature thereof at about 0°C. Approximately 160 mls of water at about pH 5 was then infused into the mixture at a rate of 100 ml/min. starting at about 0°C. Thus, a total of 250 mls of water was infused into the mixture. A Harvard Infusion pump and a 60 cc plastic syringe and 19 gauge infusion set (butterfly) were used to infuse the water. Co-precipitation occurred after about 125 mls of water were infused into the mixture and at a temperature of about 2°C. Following co-precipitation, the suspension was stabilized by adding 150 mls of about 1% polyvinylpyrrolidone (PVP) at about pH 7.4 and kept at a temperature at about 10°C. The suspension was then poured into five 250 ml centrifuge bottles (approximately 125 ml per bottle) and centrifuged at approximately 2500 rpm for about 30 min. The supernate was discharged. The precipitates remaining in the five centrifuge bottles were then repeatedly washed with approximately 125 mls of about 0.1% PVP at about pH 11 until the wash reached a pH of about 10 to extract the IDA from the particles and to form the

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ultrasmall, non-aggregated, porous, non-crystalline IDE particles. The particles were then treated with human serum albumin according to Example III and then lyophilized according to the procedure set forth in Example IV.

Example III

The IDE particles of Examples I and II were coated with human serum albumin to improve the biocompatibility of such particles.

To coat the IDE particles, an appropriate amount of the IDE suspension (e.g. 250 mg or 5 g) is pipetted into a 50 ml centrifuge tube. The tube is centrifuged at 2500 rpm for 30 min. and the supernatant discarded. To 5 g or 250 mg of the IDE porous particles, add 100 mls or 5 mls, respectively, of about 25% human serum albumin (HSA) and mix thoroughly with vortex mixer. A preferred HSA/IDE ratio in accordance with the instant invention is about 2:1 to about 5:1. Let the HSA mixture stand at room temperature for about two hours. After standing, mix thoroughly with a vortex mixer approximately every 30 min., and then store at 40°C overnight. Centrifuge the stored mixture at about 3000 rpm for 120 min. Discard supernate. Add by pipette, approximately 2.0 mls for 250 mg of IDE

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or 15 mls for 5 g of IDE of about 0.1% PVP at about pH 7.4 and mix thoroughly with vortex mixture.

Example IV

5 To lyophilize the IDE particles of Examples I or II, place centrifuge tubes in dry ice/acetone bath to freeze suspension as quickly as possible. To facilitate a thin frozen layer, the suspension may be rolled onto the lower sides of the centrifuge tube.

10 Once frozen, place frozen tubes in lyophilizer as quickly as possible and begin lyophilization. Typical lyophilizer settings include: freeze-dryer temperature equal about -55°C; shelf temperature is equal to about -40°C; and vacuum is
15 at about 10-50 micrometer mercury. The frozen suspension should be lyophilized until the moisture remaining is about 2% or less. When removing from the lyophilizer, immediately cap the centrifuge tubes and store in a refrigerator at about 5°C until
20 needed.

Alternatively, to lyophilize the IDE particles of Examples I or II, a compound such as mannitol, or other suitable agent known to one skilled in the art to facilitate lyophilization cake
25 formation, should be added to the suspending vehicle. Up to about 4 ml of suspension then is

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added to about 10 ml lyophilization vials (or equivalent ratio for larger volumes) and flash freeze the vial contents. The vials then are placed in a lyophilizer for vacuum drying. A profile that works includes a condenser of about -55°C and a vacuum of about 10-50 micrometers of mercury. The shelf temperature is adjusted to about -40°C for about 48 hours then increased to about -15°C for about 24 hours followed by about 8 hours at about 20°C. Other profiles could be acceptable as long as the moisture remaining is about 2% or less. The vials should be sealed, removed from the lyophilizer and stored at about 5°C until needed.

Example V

To prepare the 1:6 bubbles (porous particles) suspension (Example 16, Table I), about 10 grams of iodipamide ethyl ester (IDE) and about 60 grams of iodipamic acid (IDA) were added to a two liter beaker. Approximately two hundred milliliters of dimethylsulfoxide (DMSO) were added to the beaker and stirred for approximately one hour until all of the solids dissolved. About two hundred and fifty milliliters of ethanol was then added to the solution and the solution was mixed. The beaker containing the solution was then placed in a dry ice/acetone bath and stirred quickly while it cooled

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to about 10°C. Two hundred and twenty milliliters of water (about pH 5) was then infused at a rate of about 150 ml/min by means of a Harvard Infusion pump, about 60 cc plastic syringe, and 19 gauge butterfly set. The water (about pH 5) was infused in about twenty milliliter increments into the stirring beaker, allowing the temperature to return to about 10°C after each increment. The solution in the beaker was then cooled to about 8°C and about 60 milliliters of water (about pH 5) was infused at a rate of about 100 ml/min, so that the precipitation would occur at a total infused volume of about 250 ml and a temperature of about 9°C. An additional 220 ml of water (about pH 5) was then infused at a rate of about 150 ml/min into the beaker. The co-precipitated bubbles (porous particles) were then stabilized by the addition of about 300 ml of 1% PVP (about pH 7.4).

The material was then poured into six 250 ml centrifuge bottles (about 208 ml per bottle) and centrifuged at about 2500 rpm for about 45 minutes. The supernatant was discarded and each bottle was resuspended in about 125 ml of about 0.1% PVP (about pH 11). The bottles were then centrifuged (about 2500 rpm for about 30 minutes) twice more and resuspended in both about 125 ml of about 0.1% PVP (about pH 11) per bottle and finally about 50 ml of

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about 0.1% PVP (about pH 11) per bottle. About one hundred and twenty five milliliters of about 0.1 N sodium hydroxide was then added to each bottle. The bottles were then washed eleven times by
5 centrifuging at about 2500 rpm for about 30 minutes and resuspending with about 0.1% PVP (about pH 7.4). Once all of the material was washed, the material was again centrifuged and resuspended in about 0.1% PVP, about 10% mannitol (about pH 7.4),
10 diluted with the mannitol solution to about 50 mg/ml, and lyophilized in doses of 4 ml per 10 ml vial as described in Example IV.

Table I hereinafter illustrates the production of ultrasmall porous IDE particles in accordance with Examples I and V. More particularly,
15 Examples 6-10 in Table I generally follow the procedure outlined in Example I, whereas Examples 11-14 in Table I also follow Example I procedures generally, but illustrate a variation in the IDE/IDA ratio. Example 15, as compared to Example 13, in
20 Table I shows how the diameter of the porous particles can be increased by increasing temperature. Examples 15 and 16 demonstrate how porous particles production can be scaled up while
25 maintaining constant particle diameter.

TABLE I

Example No.	6	7	8	9	10	11	12	13	14	15	16
IDE (grams)	0.5	1.0	2.0	0.5	0.75	1.0	1.0	1.0	1.0	1	10
IDA (grams)	1.0	2.0	4.0	1.0	1.5	1.0	4.0	8.0	8.0	6	60
DMSO (ml)	10	20	40	5	5	20	20	20	20	20	200
ETOH (ml)	12.5	25	50	6.25	6.25	25	25	25	25	25	250
Precipitating Solution (ml)	25	50	100	12.5	12.5	50	50	50	50	50	500
Inclusion Rate (ml/min)	10	20	40	5	5	100	100	20	100	20	150
Temperature (°C)	1	0	1.5	23	24.5	6	1	-10	5	11.5	8.5
Bubble Size (nm)	584	572	619	1240	1620	488	459	571	513	1055	1019

Exs. 6-8 Demonstrate scaleup of the 1:2 porous particles preparation at the 0.5 micron diameter size.

Exs. 6,9,10 Demonstrate how particle size is increased by increasing the concentration and temperature of precipitation.

Exs. 11-14 Demonstrate varying IDE/IDA ratios while maintaining 0.5 micron diameter.

Exs. 15-16 Demonstrate scaleup of the 1:6 porous particles preparation at the 1 micron diameter size.

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The present invention may, of course, be carried out in other specific ways than those herein set forth without departing from the spirit and essential characteristics of the invention. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive and all changes coming within the mean and equivalency range of the appended claims are intended to be embraced therein.

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Having described our invention, we claim:

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5 (1) Ultrasmall, substantially non-aggregated, non-crystalline porous particles having substantial uniformity in size selected from a particle diameter range of up to about 10 microns, said particles having the ability to trap gas bubbles within its porous matrix when suspended in a liquid and to enhance medical images of blood vessels and soft tissue when introduced into the blood stream of an animal.

(2) Ultrasmall porous particles according to claim 1 wherein the mean particle diameter is selected from the range of from about 0.01 microns to about 5.0 microns.

(3) Ultrasmall porous particles according to claim 1 wherein the mean particle diameter is selected from the range of from about 0.1 microns to about 2.0 microns.

(4) Ultrasmall porous particles according to claim 1, said particles being porous iodipamide ethyl ester particles.

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(5) Ultrasmall porous particles according to claim 1, said particles being suspended in a liquid and having gas bubbles entrapped within their porous matrices.

(6) Ultrasmall porous particles according to claim 1, said particles having a coating on their surfaces.

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(7) Ultrasmall, substantially non-aggregated, non-crystalline porous particles having substantial uniformity in size selected from a particle diameter range of up to about 10 microns, said particles being produced by the process comprising:

(a) preparing a solution which includes first and second solid compounds in a suitable solvent for the two compounds wherein;

(b) infusing a precipitating liquid into the solution at a temperature between about -50°C and about 100°C at an infusion rate of from about 0.01 ml/min. to about 3000 ml/min. per 50 ml unit volume of solution, the two solid compounds having essentially little solubility in the precipitating liquid and the solvent being miscible in the precipitating liquid, so as to produce a suspension of precipitated amorphous, non-crystalline solid compounds in the form of substantially non-aggregated particles of a uniform size selected from a particle diameter range of up to about 10 microns, the particle size being directly related to the solution temperature during precipitation and inversely related to the infusion rate; and

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25 (c) washing-the particles in a suitable
washing liquid, the first compound having essen-
tially little solubility in the washing liquid and
the second compound being soluble in said washing
liquid, so that the second compound is extracted
from the first compound to produce the ultrasmall,
30 substantially non-aggregated, non-crystalline porous
particles.

(8) Ultrasmall porous particles according to
claim 7, said process further including the step of:
separating the co-precipitated particles
from the solvent before said washing or during said
5 washing.

(9) Ultrasmall porous particles according to
claim 7, wherein additional precipitating liquid is
added to the suspension before the particles are
washed.

(10) Ultrasmall porous particles according to
claim 8, wherein the particles are separated by
centrifugation, membrane filtration, or reverse
osmosis.

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(11) Ultrasmall porous particles according to claim 7, wherein the precipitating liquid is a surfactant solution.

(12) Ultrasmall porous particles according to claim 7, wherein a solution is prepared such that the concentration of the two solid compounds are near their solubility limits in the solvent.

5 (13) Ultrasmall porous particles according to claim 7, wherein the first solid compound is iodipamide ethyl ester and the second solid compound is iodipamic acid, the solvent is DMSO, the precipitating liquid is water at a pH of about 5.0, and the washing liquid is an aqueous solution of polyvinylpyrrolidone at a pH of about 11.

(14) Ultrasmall porous particles according to claim 7, wherein the mean particle diameter is selected from the range of from about 0.01 microns to about 5.0 microns.

(15) Ultrasmall porous particles according to claim 7, wherein the mean particle diameter is selected from the range of from about 0.1 microns to about 2.0 microns.

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(16) Ultrasmall porous particles according to claim 7, wherein the particle size distribution has a maximum relative standard deviation of about 40%.

(17) Ultrasmall porous particles according to claim 7 wherein the weight ratio of the second compound soluble in the washing fluid to the first compound insoluble in the washing fluid is in the
5 range of from about 2:1 to about 10:1.

(18) Ultrasmall porous particles according to claim 7, said process including the further step of drying said ultrasmall porous particles.

(19) Ultrasmall porous particles according to claim 7, said ultrasmall porous particles being suspended in a liquid and having gas or a medically useful compound entrapped within their porous
5 matrices.

(20) Ultrasmall porous particles according to claim 7, said process including the further step of coating said ultrasmall porous particles.

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(21) A sterile injectible fluid composition of matter in unit dosage form and adapted for injection into the blood stream of an animal for providing gas bubble echogenicity for blood vessels and soft tissue ultrasound imaging, said composition comprising a suspension of said ultrasmall porous particles as recited in claim 1 suspended in a carrier liquid which is non-toxic and physiologically acceptable and in which the ultrasmall porous particles are at least temporarily stable.

(22) A composition of matter according to claim 21 wherein the ultrasmall porous particles are iodipamide ethyl ester particles.

(23) A composition of matter according to claim 22 wherein the iodipamide ethyl ester particles are substantially uniform in size having a mean particle diameter size of about 0.5 microns.

(24) A composition of matter according to claim 21 wherein said ultrasmall porous particles are coated.

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(25) A method for enhancing ultrasound imaging in the blood vessels and soft tissue of an animal, said method comprising

5 introducing the composition of matter as recited in claim 21 into an animal in an effective amount; and

 scanning the animal with an ultrasound scanner to generate an ultrasound image.

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(26) A method of forming ultrasmall, substantially non-aggregated, non-crystalline porous particles having substantial uniformity in size selected from a particle diameter range of up to
5 about 10 microns, said method comprising:

(a) preparing a solution which includes first and second solid compounds in a suitable solvent for the two compounds;

(b) infusing a precipitating liquid into
10 the solution at a temperature between about -50°C and about 100°C at an infusion rate of from about 0.01 ml/min. to about 3000 ml/min. per 50 ml unit volume of solution, the two solid compounds have an essentially little solubility in the precipitating
15 liquid and the solvent being miscible in the precipitating liquid, so as to produce a suspension of precipitated amorphous, non-crystalline solid compounds in the form of substantially non-aggregated particles of a uniform size selected
20 from a particle diameter range of up to about 10 microns, the particle size being directly related to the solution temperature during precipitation and inversely related to the infusion rate; and

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25 (c) washing the particles in a suitable
washing liquid, the first compound having essen-
tially little solubility in the washing liquid and
the second compound having greater solubility in
said washing liquid, so that the second compound is
30 extracted from the first compound to produce the
ultrasmall, substantially non-aggregated,
non-crystalline porous particles.

(27) A method according to claim 26 including
the further step of separating the co-precipitated
particles from the solvent before said washing.

(28) A method according to claim 26 including
the further step of drying said ultrasmall porous
particles.

(29) A method according to claim 26 including
the further step of coating said ultrasmall porous
particles.

(30) A method according to claim 26 including
the further step of suspending the ultrasmall porous
particles in a liquid.

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(31) A method according to claim 26 including the further step of introducing a gas or a medically useful compound into said ultrasmall porous particles.

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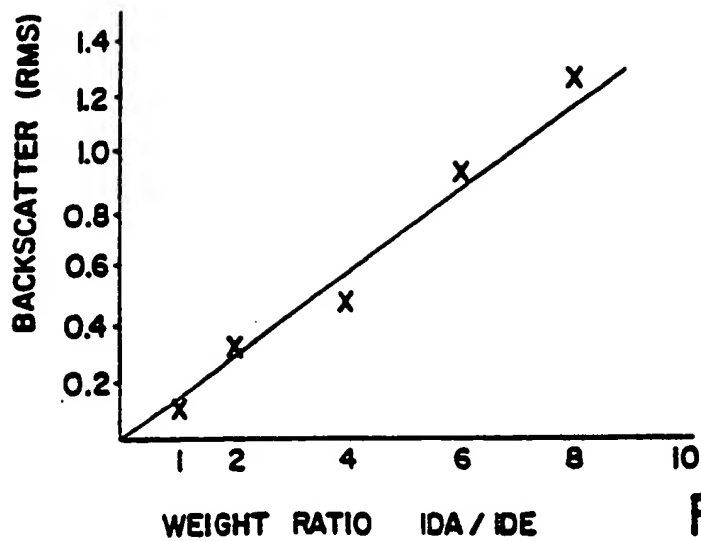


FIG. 1

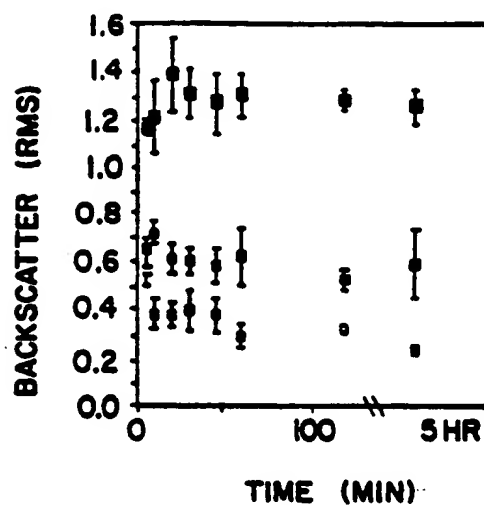


FIG. 2

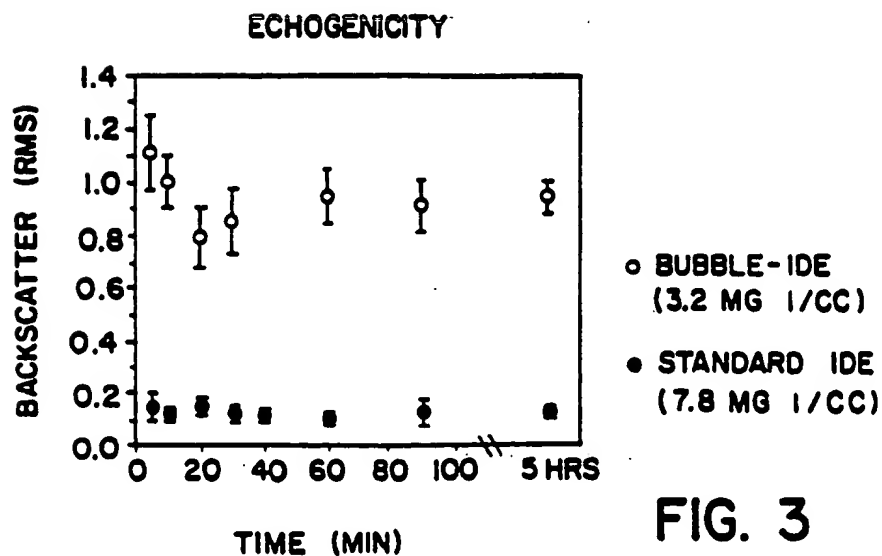


FIG. 3

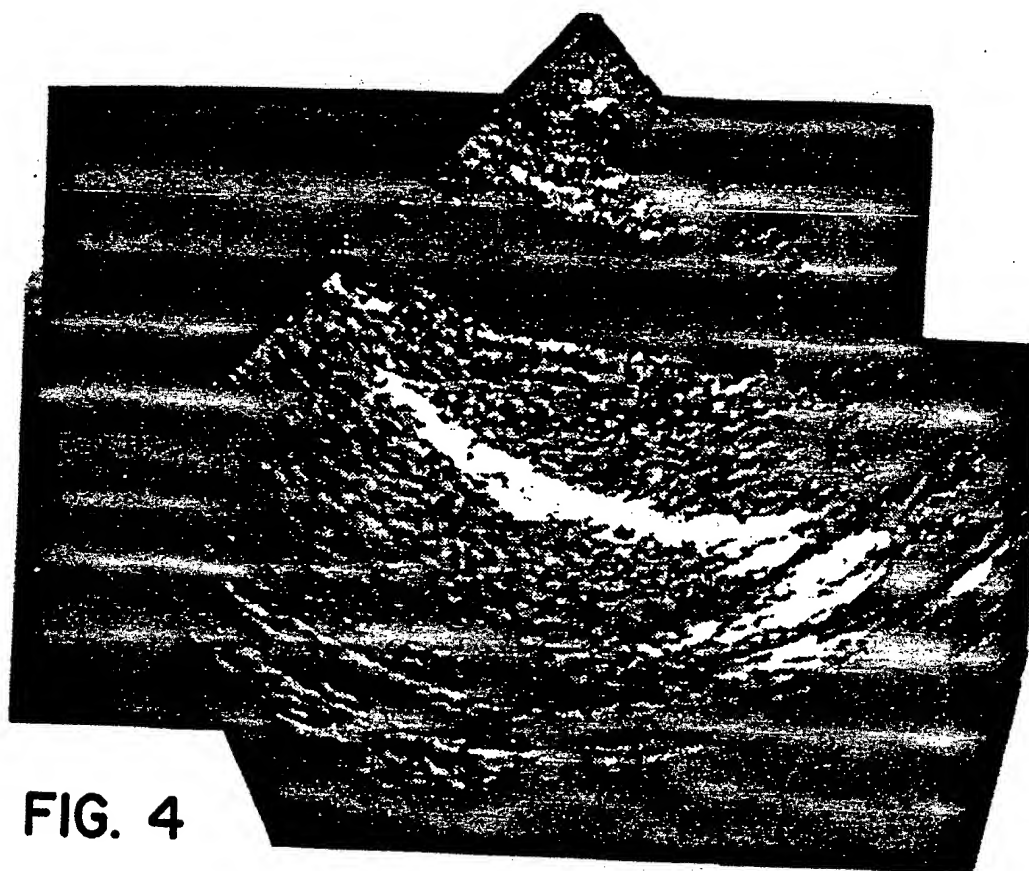


FIG. 4

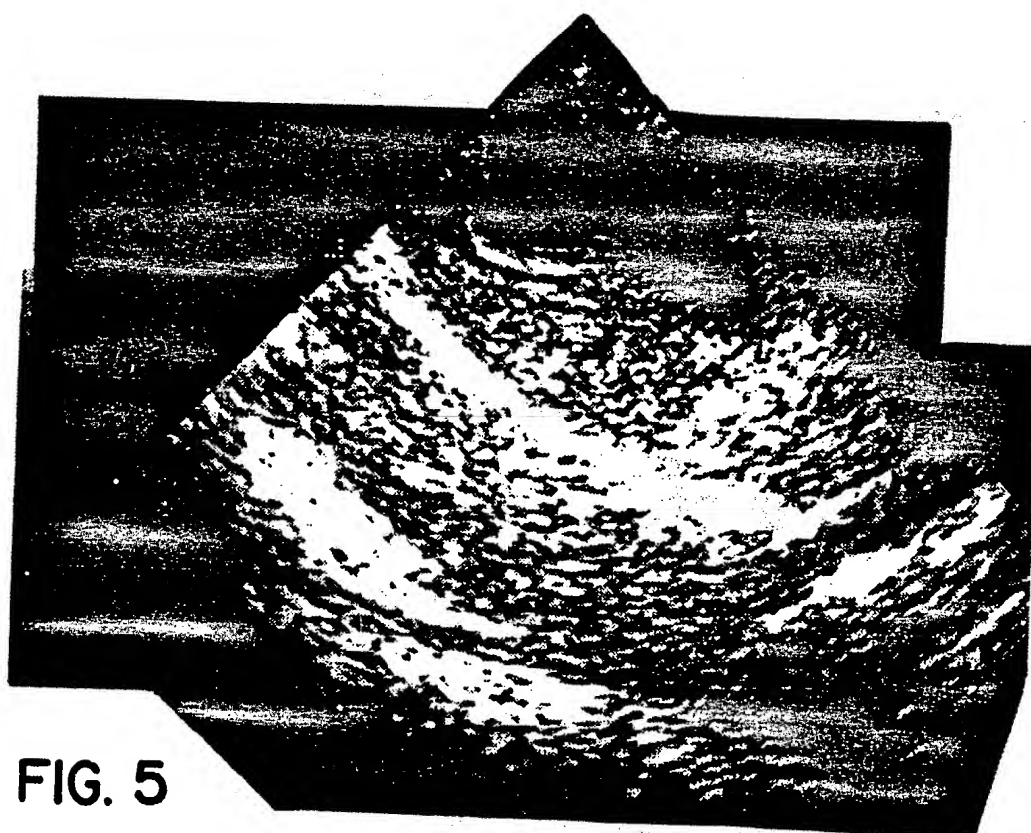


FIG. 5

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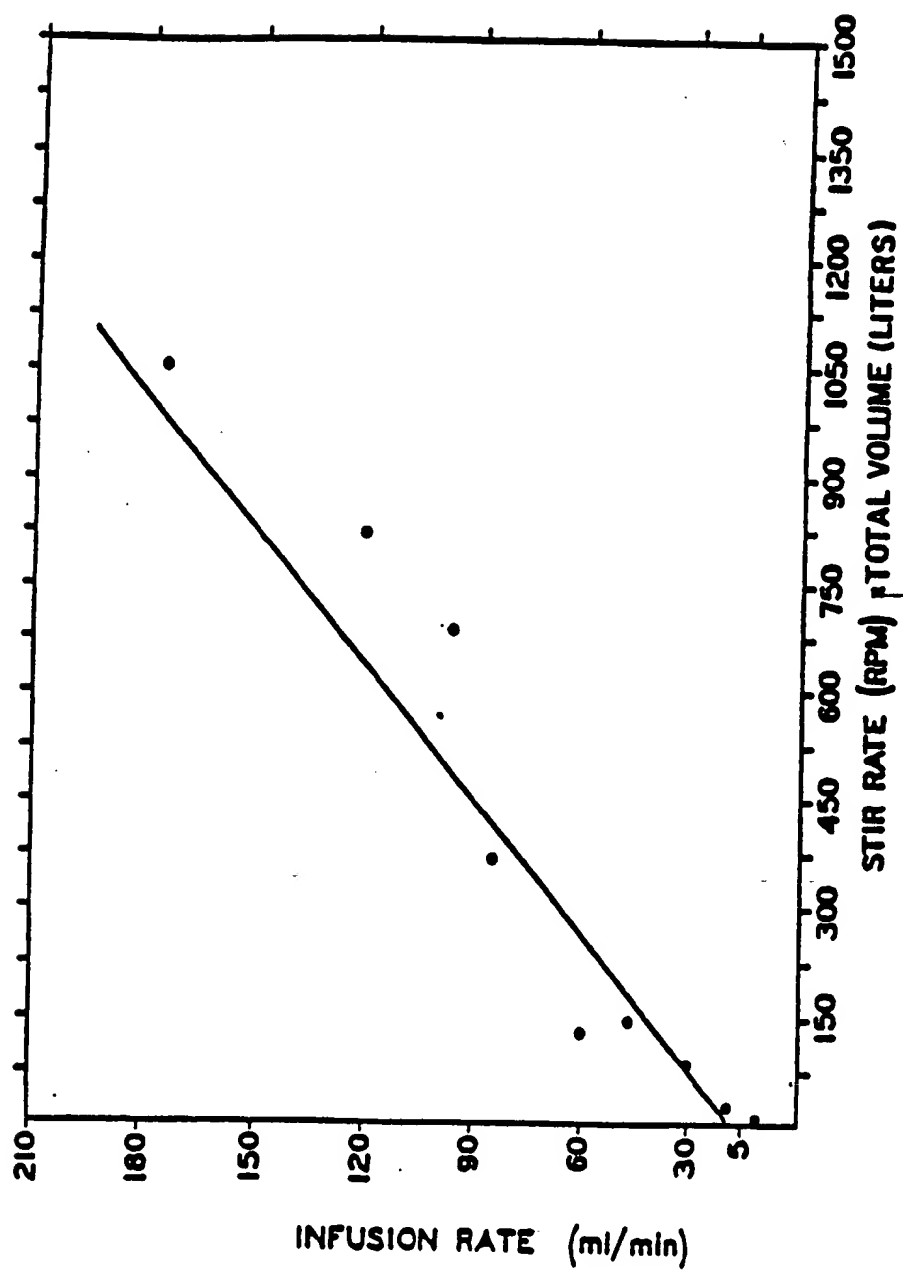


FIG. 6

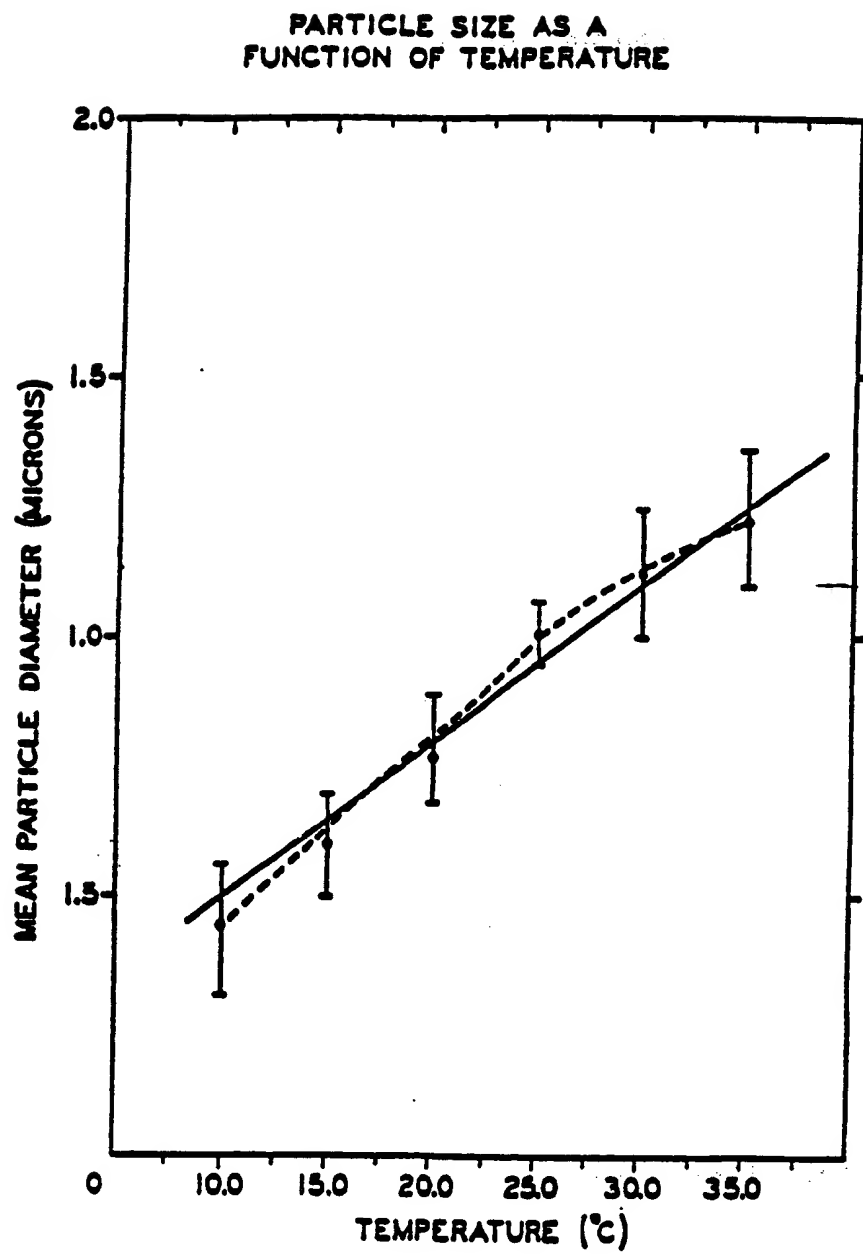
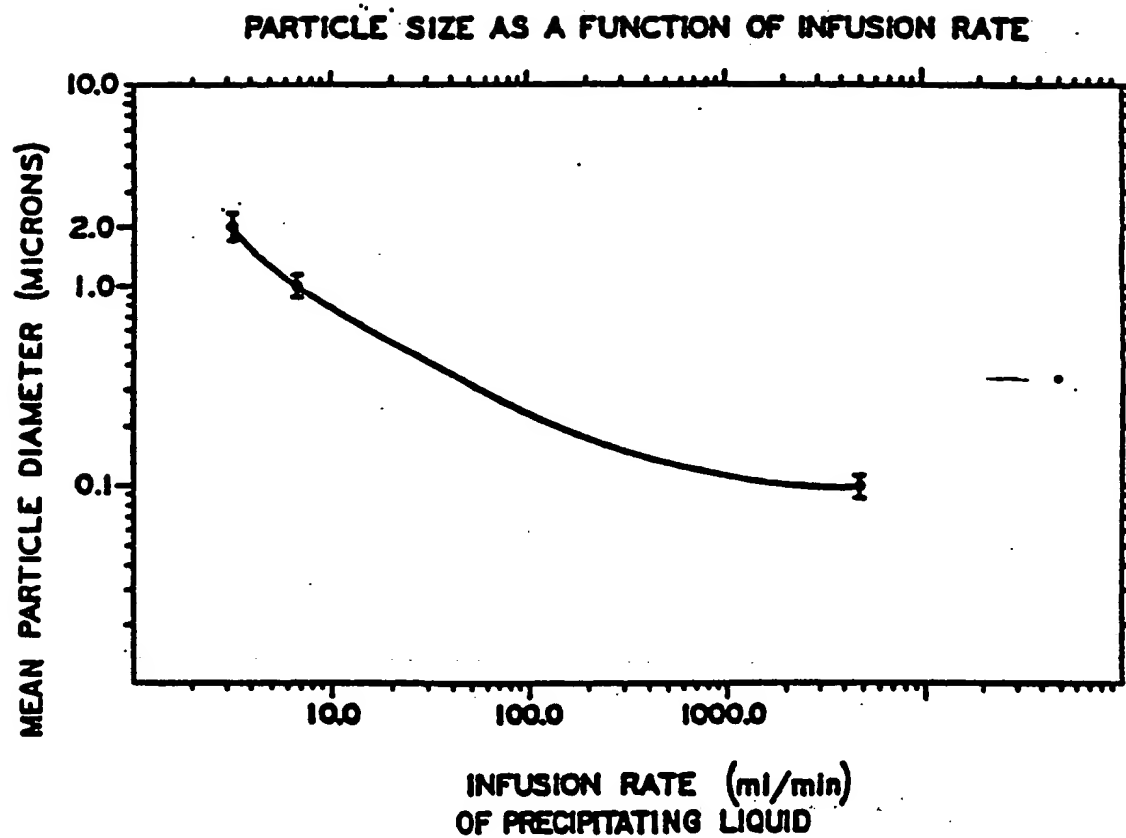


FIG. 7

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all) ⁶		
According to International Patent Classification (IPC) or to both National Classification and IPC.		
Int.C1.5	A 61 K 49/00	A 61 K 49/04
II. FIELDS SEARCHED		
Minimum Documentation Searched ⁷		
Classification System	Classification Symbols	
Int.C1.5	A 61 K	
Documentation Searched other than Minimum Documentation to the extent that such Documents are included in the Fields Searched ⁸		
III. DOCUMENTS CONSIDERED TO BE RELEVANT⁹		
Category ¹⁰	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
Y	EP,A,0169618 (STERILIZATION TECHNICAL SERVICES) 29 January 1986, see page 4, line 23 - page 5, line 19; page 6, lines 1-8; table If; page 29, example 29	1-31
Y	EP,A,0052575 (ULTRA MED. INC.) 26 May 1982, see page 10, lines 11-28; page 41, line 9 (cited in the application)	1-31
Y	EP,A,0414287 (NYCOMED S.A.) 27 February 1991, see page 3, line 28 - page 4, line 10; page 4, lines 34-39, 51-56 -/-	1-31
<p>* Special categories of cited documents: ¹⁰</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"A" document member of the same patent family</p>		
IV. CERTIFICATION		
Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report	
23-09-1992	02.11.92	
International Searching Authority	Signature of Authorized Officer	
EUROPEAN PATENT OFFICE	Dagmar Frank	

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category *	Character of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
P,X	STN File Server, File Medline, AN=92225855, M.R. VIOLANTE et al.: "Particle-stabilized "bubbles" for enhanced organ ultrasound imaging", & INVEST. RADIOL., (1991 NOV) 26 SUPPL. 1 S194-7, DISCUSSION S198-200, see whole abstract -----	1-31
A	DE,A,3246386 (M.W. HELZEL) 20 June 1984, see claims -----	1-31

**FIG. 8**

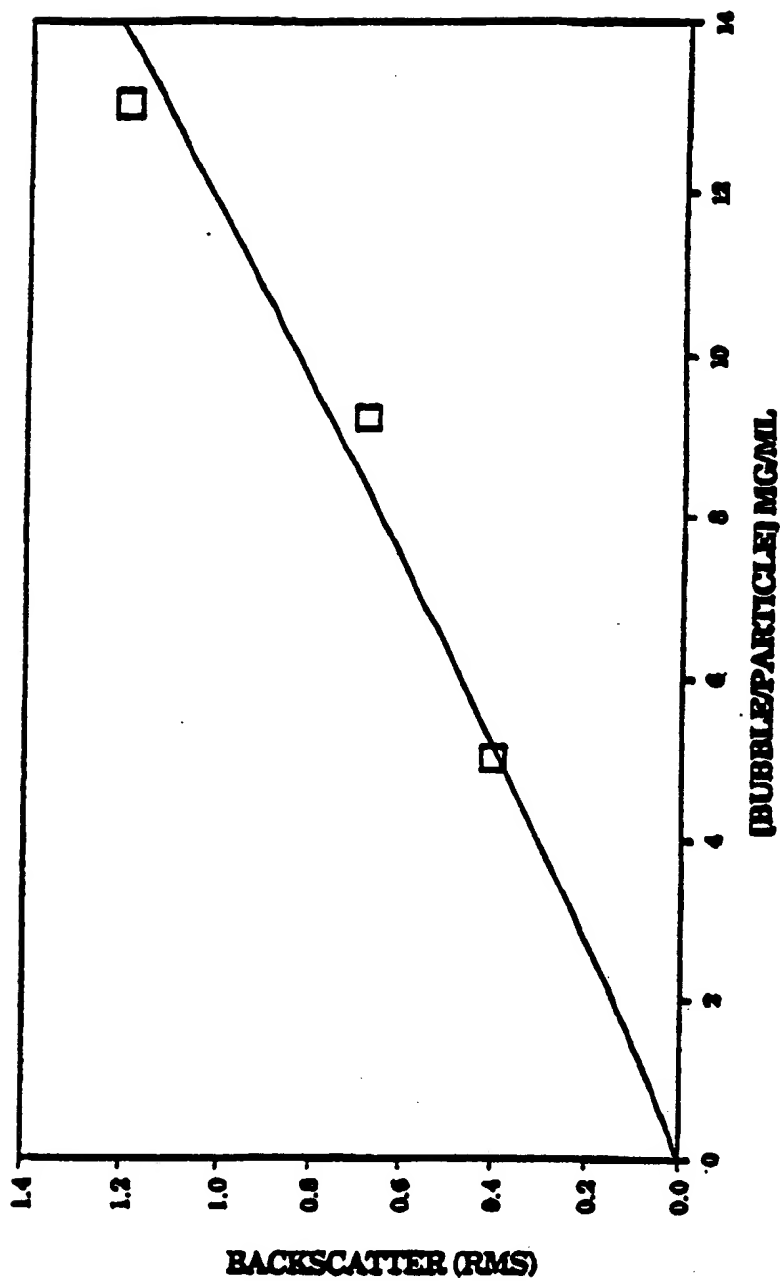


FIG. 9 !

**ANNEX TO THE INTERNATIONAL SEARCH REPORT
ON INTERNATIONAL PATENT APPLICATION NO.**

US 9205592

SA 62092

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 20/10/92. The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

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		EP-A- 0483278	06-05-92
DE-A- 3246386	20-06-84	None	

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